IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:		**Docket No.:**	**020884.000009**
Applicant(s):	**Jose Vicente Castell Ripoll, *et al.***	**Examiner:**	**QIAN, Celine X**
Application No.:	**10/597,286**	**Art Unit:**	**1636**
U.S. Filing Date:	**October 20, 2006**	**Conf. No.:**	**8850**
Title:	**METHOD FOR OBTAINING A SINGULAR CELL MODEL CAPABLE OF REPRODUCING IN VITRO THE METABOLIC IDIOSYNCRASY OF HUMANS**	**Customer No.:**	**24239**

DECLARATION UNDER 37 CFR §1.132 IN U.S. PATENT APPLICATION NO. 10/597,286

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

José Vicente Castell Ripoll hereby declares:

1. THAT I am a co-inventor of the subject matter disclosed and elected in United States Patent Application No. 10/597,286 having a filing date of October 20, 2006 in the names of José Vicente Castell Ripoll, Ramiro Jover Atienza, Agustín Lahoz Rodríguez, and Maria José Gómez-Lechón and entitled, "**METHOD FOR OBTAINING A SINGULAR CELL MODEL CAPABLE OF REPRODUCING IN VITRO THE METABOLIC IDIOSYNCRASY OF HUMANS**," hereafter referred to as the "Application."

2. THAT the Application relates, in general, to obtaining a singular cell model capable of reproducing *in vitro* the metabolic idiosyncrasy of humans by expression vectors that encode for the sense and anti-sense mRNA of the enzymes of the drug biotransformation Phases I and II showing greatest variability in humans. The claims being examined specifically relate to a method for obtaining a cell model as recited in claim 1:

1. A method for obtaining a cell model capable of reproducing *in vitro* a metabolic idiosyncrasy of humans, wherein said model comprises a set of recombinant adenoviral expression vectors that confer to transformed cells a phenotypic profile of drug biotransformation enzymes designed at will, in order to reproduce the metabolic idiosyncrasy of humans, said method comprising:

a) Transforming human cells of hepatic origin expressing cytochrome P450 reductase with a set of more than one recombinant adenoviral expression vectors comprising an ectopic DNA sequence that codes for drug biotransformation enzymes selected from among Phase I drug biotransformation enzyme and Phase II drug biotransformation enzyme,

wherein each expression vector comprises an ectopic DNA sequence that codes for a different Phase I or Phase II drug biotransformation enzyme, selected from the group consisting of:

(i) a DNA sequence transcribed in the sense mRNA of a Phase I or a Phase II drug biotransformation enzyme ("sense vector") ; and

(ii) a DNA sequence transcribed in the anti-sense mRNA of a Phase I or a Phase II drug biotransformation enzyme ("anti-sense vector");

wherein the expression of said ectopic DNA sequences in the cells transformed with one or more of the aforementioned expression vectors confers on the transformed cells specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes,

to obtain cells that transitorily express said ectopic DNA sequences, and

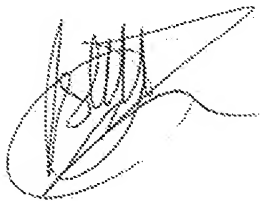
b) building a cell model capable of reproducing *in vitro* the metabolic idiosyncrasy of humans from said cells transformed with the expression vectors so that the result is the expression of any phenotypic profile of Phase I or Phase II drug biotransformation enzymes desired.

3. THAT in the March 15, 2010 Final Office Action, claim 1, and the claims depending therefrom, were rejected under 35 U.S.C. §103(a) as being unpatentable over Bort et al. (*Biochem. Pharmacol.*, 58(5), 787-796 (1999)) (hereinafter Bort) in view of Gómez-Lechón et al. (*Curr. Drug Metabolism*, 4(4), 292-312 (2003)) (hereinafter Gomez-Lechon).

4. THAT Exhibit A attached herewith is an experimental report prepared in support of the instant application. As disclosed in the experimental report, the method provides specific and individual control of expression of ectoptic DNA sequences so that specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes can be conferred on transformed cells. Further, the method is able to reproduce *in vitro* the metabolic idiosyncrasy of humans with better results than the imprecise method discussed in Gomez-Lechon.

5. THAT I offer Exhibit A with this Declaration as evidence of the non-obviousness of claims 1 – 9, 11, and 13 – 19, specifically that the method of the instant application provides precise control over the expression of ectopic DNA conferring on the transformed cell specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes compared to the method discussed in Gomez-Lechon.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Date: 20 July 2010

José Vicente Castell Ripoll

EXHIBIT A

EXPERIMENTAL REPORT

Introduction

In the present report we show that a co-expressing model which employs more than one of the expression vectors encoding for different CYPs allows one to specifically modulate the expression of the enzymes in a simple manner, giving the possibility to mimic the *in vivo* human metabolic capabilities of hepatocytes. Significantly, the enzymatic activities obtained can be controlled to be similar or even higher than that found in human hepatocytes cultures.

Further, in a comparative example with Gómez-Lechón et al., we show that using the method of the invention, the enzymatic activities obtained are comparable with that found in human hepatocytes cultures, whereas in Gómez-Lechón et al., although there is an increase in the expression levels, no correlation was found between mRNA levels and CYP activities, in the best scenario the enzymatic activity is nearly 1.000-fold lower than that found in human hepatocytes culture.

1. Mimic *in vitro* human hepatocyte metabolic capabilities in non-competent cell lines by transfection with recombinant adenovirus cocktail.

Method outline

Adenoviruses can rapidly and efficiently infect human cells (hepatocytes and hepatoma cell lines) and, by using an appropriate multiplicity of infection (MOI), almost 100% of the cells can express the transgene after a short exposure to the virus, and a functional level of transgene expression can be achieved without major changes in the expression of constitutive or inducible hepatic genes.
Adenoviral vectors encoding for major drug biotransforming CYP genes were constructed as described in the present patent application. Human hepatoma HepG2 cells were plated in Ham's F-12/Leibovitz L-15 (1/1, v/v) supplemented with 7% newborn calf serum and cultured to 70% confluence. Cells were infected with the recombinant adenovirus for different periods of time (2-24h) at a multiplicity of infection (MOI) ranging from 1 to 200. Thereafter, cells were washed and fresh medium added. To determine the metabolic activity of transfected human hepatoma cells specific CYP activities were measured 48 h after adenoviral infection. Enzymatic activities were measured by HPLC-MS/MS by using several selective substrates as describe in the literature.

Results

Initial results demonstrated that Ad-CYP vectors significantly increased the metabolic competence of human hepatoma cells; such increase was about to be linear to the amount of virus used (MOIs). Co-infection with different expression vectors was carried out by following the developed protocol for single infection. Therefore, HepG2 cells were infected with different mixtures of the constructed expression vectors.

As an example, **Figure 1** shows the potential of our cell model to mimic an *in vitro* human hepatocyte profile. Here the activity of three of the most relevant human CYP450 in a human hepatocyte culture is compared with the infected HepG2 hepatoma cell line. The infection was carried out with each of the specific expression vectors and with a mixture of them. As shown when comparing profile **A** versus profile **F**, it has been possible to provide metabolic human capabilities to a non-metabolic competent cell line such as HepG2 and to mimic the enzymatic activity of a human hepatocyte culture. Further, up to five recombinant adenoviruses have been used to co-express five different CYP450 enzymes (data not shown).

An additional feature of our cell model is the possibility to reach even higher enzymatic activities than that found in human hepatocytes cultures (**Figure 2**). This fact allows the model to mimic different idiosyncratic situations as for instance enzymatic induction by drug co-administration, polymorphism etc.

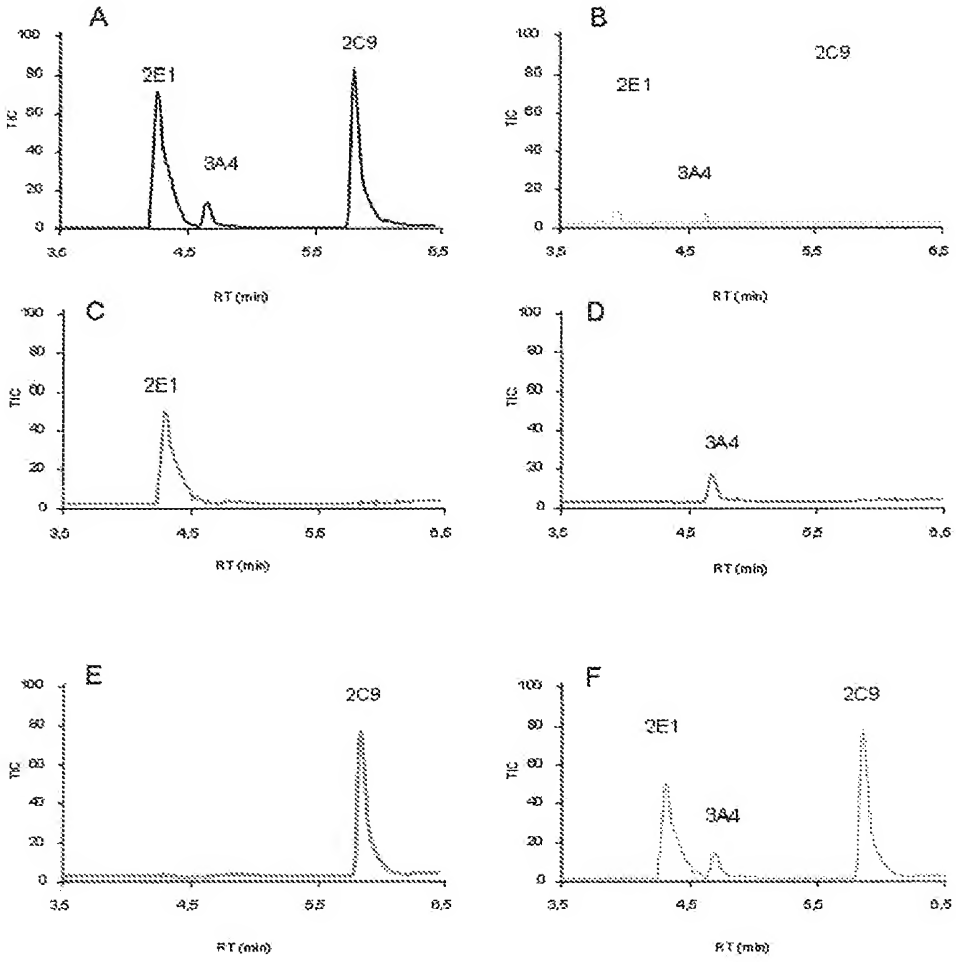


Figure 1. Reconstructed total ion chromatograms of HPLC/MS-MS of the CYP 2E1, 3A4 and 2C9 activities profile. **A.** Human hepatocyte culture. **B.** Non metabolic competent cell line (HepG2) culture. **C.** CYP 2E1 over-expression in HepG2. **D.** CYP 3A4 over-expression in HepG2. **E.** CYP 2C9 over-expression **F.** CYP 2E1, 3A4 and 2C9 co-expression in HepG2 by using vectors mixture.
CYP450 3A4 activity was measured by midazolam hydroxylation, 2E1 by chlorzoxazone hydroxylation and 2C9 by diclofenac hydroxylation. All the hydroxylated metabolites were measured by HPLC-MS/MS in multiple reaction monitoring (MRM) mode.

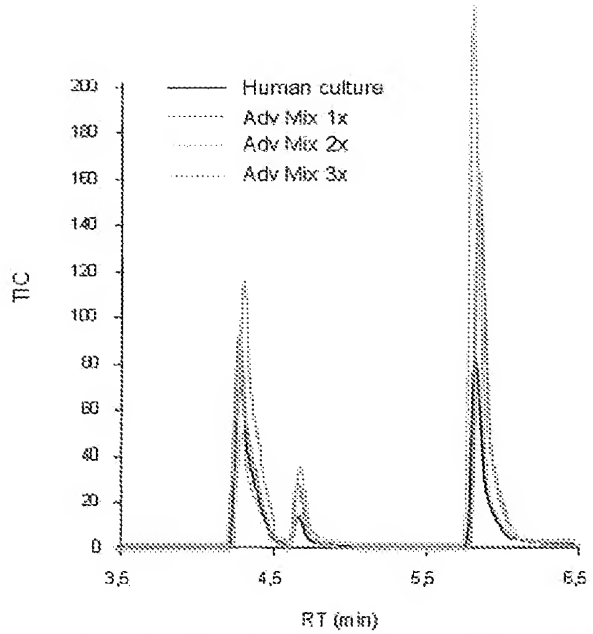


Figure 2. Reconstructed total ion chromatograms of HPLC/MS-MS of the CYP 2E1, 3A4 and 2C9 activities profile. Infections with increasing MOI allow controlled over expression of CYP activities, reaching even higher enzymatic activities than that found in human hepatocyte culture.

2. Comparative example with respect to Gómez-Lechón

Studies of typical hepatic genes have shown that liver-gene expression is accomplished by the concerted action of a small number of "liver enriched transcription factors" (LEFTs). Gomez Lechón et al. (2003) suggested the use of recombinant adenoviruses expressing LEFTs as a tool to convert non-metabolic competent cells into cells able to transiently express CYPs genes. In Gomez Lechón et al., two vectors encoding for two transcription factors (HNF3 , C/EBP) were constructed to attempt to regulate CYPs genes expression in non metabolic competent cell lines. The mRNA levels of some CYPs increased, even up to about 60-fold, see Gomez Lechón et al., figure 7 and page 307, right-hand column, lines 32-34.

However, when compared with human hepatocyte culture and with our cell models, such the transformed cell expressing CYP3A4 (disclosed in example 2 and fig 3 of the present patent application), the difference is very significant (See **Figure 3** herein). When comparing a cell model according to Gomez Lechón et al. with human activities, 1000- fold less CYP3A4 activity was found.

Consequently, infection with adenovirus encoding key transcription factors alters the expression (mRNA), but not the function of hepatic drug-metabolizing enzymes. By contrast, the use of recombinant adenovirus encoding for CYPs allowed reaching the *in vivo* human activities as can be seen in **Figure 3.**

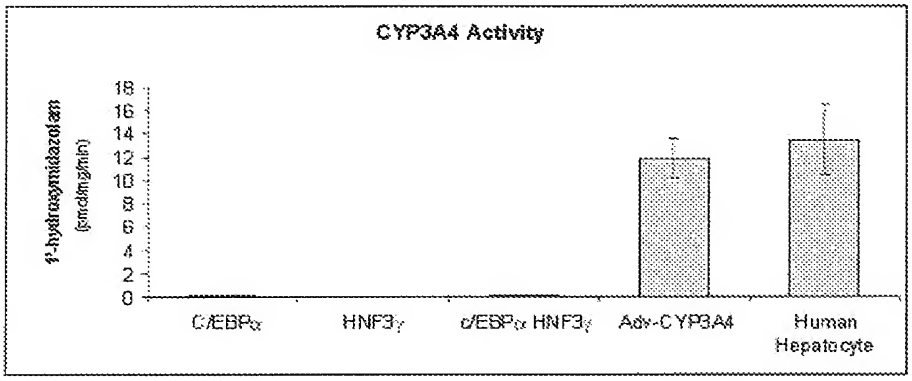


Figure 3. Comparative bar chart showing the different CYP3A4 activities in HepG2i transfected with C/EBPα vector (10 MOI), HepG2i transfected with HNF3γ vector (10 MOI), HepG2 transfected with a mixture of both C/EBPα and HNF3γ vector, HepG2 transfected with Adv-CYP3A4 vector and human hepatocyte culture. CYP3A4 activity was measured as midazolam hydroxylation by HPLC-MS/MS.